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Morphological and Mineral Characteristics of Peripheral Blood in Female Polar Fox in Relation to Age

Anna PIOTROWSKA, Roman SZYMECZKO, Małgorzata OŻGO, Monika BOGUSŁAWSKA-TRYK and Katarzyna BURLIKOWSKA

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The aim of the study was to determine the values of selected haematological and biochemical parameters in peripheral blood of female polar fox in relation to the age of the animals. The research involved 50 polar fox females three months after the lactation period (i.e. the non-mating period). Animals were divided into 5 age groups (n=10), ranging from 1 to 5 years of age. In blood samples the following parameters were determined: RBC, Ht, Hb, WBC, PLT, red blood cell parameters (MCV, MCH, MCHC) and the percentage of respective kinds of white blood cells in the total number of leukocytes. The content of Ca, Pi, Na, K, Cl, Mg, Fe, Cu and Zn as well as the ALP and ACP activity was determined in blood serum. In comparison with one year-old females, in peripheral blood of females from the remaining age groups an increase in RBC, Ht and Hb content was observed as well as a significant ($P<0.05$) decrease in WBC level together with a lower number of lymphocytes and an increase in the relative content of granulocytes and monocytes. No distinct relationship between the content of Ca, Na, Cl, Mg, Cu, Zn, the activity of ALP and ACP and the age of the animals was observed. The highest concentration of Pi and K was found in the blood serum of one year-old females. The content of Fe decreased with age and was lowest in 5 year-old animals ($P<0.05$).

Key words: Polar fox, age, blood parameters.

Anna PIOTROWSKA, Roman SZYMECZKO, Monika BOGUSŁAWSKA-TRYK, Katarzyna BURLIKOWSKA, University of Technology and Life Sciences in Bydgoszcz, Faculty of Animal Breeding and Biology, Department of Animal Physiology, Mazowiecka 28, 85-084 Bydgoszcz, Poland.
E-mail: piotrowska@utp.edu.pl
Małgorzata OŻGO, University of Agriculture in Szczecin, Faculty of Biotechnology and Animal Husbandry, Department of Animal Physiology and Biotechnology, Doktora Judyma 6, 71-466 Szczecin, Poland.

The polar fox (*Alopex lagopus* L.) attains sexual maturity at the age of 9-11 months, after completion of growth and somatic development. The life span of polar foxes is up to 10 years. However, for breeding purposes, the animals are used for no more than 4-6 years (BERESTOV & KOZHEVNIKOVA 1989). The profitability of fox breeding depends mainly on the number of born and weaned kits, which in turn depends on the condition and health state of females from the reproductive stock.

In veterinary practice, the indicator of the state of animal health are the values of haematological and biochemical indices of the blood. Blood parameters within a species are characterised by large dynamics dependent on several factors such as the season, the state of nourishment of the organism, the physiological state, the influence of stress factors, breed, sex and age of the animals (BENN *et al.* 1986; MAINKA 1988; CROOKS *et al.* 2000; ALTUNOK *et al.* 2001; TRYLAND *et al*. 2002; CROOKS *et al.* 2003; SWANSON *et al*. 2004). In farm breeding, when standard living conditions are maintained, the physiological condition, sex and age of the animals all seem to be factors that have the largest influence on blood parameters. The available literature does not contain any data concerning the values of haematological and biochemical blood parameters of polar foxes in relation to these factors.

The aim of the study was to determine the values of selected haematological parameters, the content of mineral components and the activity of selected enzymes in peripheral blood of female polar fox in relation to the age of the animals in the non-mating period.

Material and Methods

Experimental animals

The research involved fifty clinically healthy females of the polar fox in the non-mating period, three months after the lactation period. The examined animals came from a domestic reproductive farm. Females which had raised a similar number of puppies in a litter (from 7 to 10), were divided into 5 age groups (n=10): one-year-old females (group I), two-year-old females (group II), three-year-old females (group III), four-year-old females (group IV) and five-year-old females (group V). The animals were fed on standard diets used on the farm for feeding the reproductive animals in this period. The diet consisted of animal offals, meat-and- bone meal, rapeseed oil, extruded cereals and a minerals and vitamins mixture. The content of metabolizable energy (ME) in 1 kg of the diet amounted to 1700 kcal. The distribution of ME from protein, fat and carbohydrates was respectively: 29, 54 and 17% and was in agreement with metabolizable energy requirements set for a reproductive stock of polar foxes in the non-mating period (HANSEN 1992).

Chemical analysis

Blood for analysis was taken in the morning (8.00-11.00), prior to animal feeding by puncture of a brachial artery. Blood samples were put in tubes with EDTA as anticoagulant. The following parameters were determined with the use of a Bayer Advia 120 haematology analyzer: red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit index (Ht), white blood cell count (WBC) and the number of thrombocytes (PLT). Also, the values of red blood cell parameters were defined: mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), as well as the percentage of particular kinds of white blood cell in the total number of leukocytes. In blood serum samples stored at -20°C, the content of total calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg) and iron (Fe) was determined by the spectrophotometric method and the content of sodium (Na), potassium (K) and chlorides (Cl) was determined with the use of ionoselective electrodes. The activity of total alcalic phosphatase (ALP) was determined by the spectrophotometric method. All the above mentioned parameters were determined with the help of an Advia 1650 analyzer using original Bayer kits. The total acidic phosphatase (ACP) activity was determined by an immunoenzymatic method with the use of a bioMerieux Conelab biochemical analyzer. The content of copper (Cu) and zinc (Zn) in the blood serum samples were determined by atomic absorption spectrometry (AAnalyst 400, PerkinElmer).

Statistical analysis

The obtained results were subjected to an analysis of variance using the Statistica 5.5 PL software. Differences were considered significant at $P<0.05$.

Results and Discussion

Table 1 presents the results of haematological indices in the blood of female polar foxes from reproductive stock in relation to age in the non-mating period. The erythrocyte count, the

Table 1

Haematological indices in the blood of polar fox females ($\bar{x} \pm$ SD); a, b, c – means in the rows with different letters differ significantly ($P<0.05$)

Parameters	Group				
	I	II	III	IV	V
Erythrocytes, $T \cdot l^{-1}$	$8.56^{a} \pm 0.24$	$8.94^{a,b} \pm 0.50$	$8.66^{a} \pm 0.34$	$9.18^{b} \pm 0.48$	$8.84^{a,b} \pm 0.61$
Haematocrit, $l \cdot l^{-1}$	$0.48^{a} \pm 0.02$	$0.51^{b,c} \pm 0.03$	$0.49^{a,c} \pm 0.02$	$0.52^{b} \pm 0.02$	$0.49^{a,c} \pm 0.04$
Haemoglobin. $g \cdot dl^{-1}$	$15.40^{a} \pm 0.53$	$16,22^{b,c} \pm 0.85$	$15.55^{a,c} \pm 0.61$	$16.51^{b} \pm 0.67$	$15.53^{a,c} \pm 0.97$
MCV, fl	55.89 ± 2.11	56.78 ± 0.91	56.82 ± 2.20	55.77 ± 1.93	55.56 ± 1.63
MCH, pg	17.98 ± 0.57	18.17 ± 0.51	17.94 ± 0.69	17.92 ± 0.59	17.59 ± 0.62
MCHC, $g \cdot dl^{-1}$	32.16 ± 0.32	31.99 ± 0.71	31.57 ± 0.56	32.13 ± 0.61	31.68 ± 0.51
Leucocytes, $G \cdot l^{-1}$	$13.09^{a} \pm 2.13$	$8.93^{b} \pm 2.96$	$8.97^{b} \pm 1.89$	$8.23^{b} \pm 1.86$	$9.11^{b} \pm 2.55$
Lymphocytes, %	$69.89^{a} \pm 8.68$	$45.10^{b} \pm 12.49$	$50.60^{b} \pm 17.51$	$41.90^{b} \pm 9.90$	$40.22^{b} \pm 16.48$
Granulocytes, %	$28.89^{a} \pm 8.59$	$50.80^{b} \pm 11.74$	$46.10^{b} \pm 17.19$	$55.30^{b} \pm 9.65$	$56.56^{b} \pm 15.88$
Monocytes, %	$1.22^{a} \pm 0.97$	$4.10^{b} \pm 2.02$	$3.30^{b} \pm 1.89$	$2.78^{ab} \pm 1.87$	$3.22^{b} \pm 1.48$
Thrombocytes, $G \cdot l^{-1}$	541.89 ± 76.72	443.80 ± 52.21	519.00 ± 127.08	484.10 ± 45.71	479.78 ± 43.48

haematocrit value and the content of haemoglobin in the blood of experimental females were within or close to reference values determined for adult individuals of this species (BERESTOV & BRANDT 1989). However, there were some differences in values of these parameters in relation to age. In comparison with one-year-old females, the blood of females from the remaining age groups was characterised by increased RBC, Ht and Hb, while the highest values of the analysed parameters were found in four-year-old females. The content of RBC, the Ht value and the Hb concentration in the blood of group IV females were respectively: 9.18 $T \cdot l^{-1}$, 0.52 $l \cdot l^{-1}$, 16.51 $g \cdot dl^{-1}$ and they were significantly ($P<0.05$) higher in comparison with values of these parameters in the blood of one-year-old females (8.56 $T \cdot l^{-1}$, 0.48 $l \cdot l^{-1}$, 15.40 $g \cdot dl^{-1}$, respectively). The values of red blood cell parameters (MCV, MCH, MCHC) did not show any significant, age-dependent differences. The available literature does not contain any data concerning the values of haematological parameters in the blood of carnivorous fur bearing animals in relation to age and the subsequent reproductive season. Studies conducted on various species of carnivorous animals showed differences in haematological indices values in relation to age, however, in the majority of the studies, the differences concerned two age groups: young and still growing (up to the 1st year of life) and adult animals, after reaching somatic maturity (over 1 year old) (STANKIEWICZ 1973; SMITH & RONGSTAD 1980; BERESTOV & BRANDT 1989; CROOKS *et al*. 2000). The experiment of CROOKS *et al.* (2000) on wild Californian foxes showed significantly higher ($P<0.05$) red blood cell parameter values and a higher number of erythrocytes and haemoglobin content in the blood of adult animals compared to pups (less than one year old). Similar relationships were found by SMITH and RONGSTAD (1980) in young and adult wild coyotes. Also studies conducted on growing (up to the 1st year of life) and senior dogs (over 9 years old) showed a greater number of RBC as well as a significantly higher ($P<0.05$) Hb concentration and MCH value in the blood of old dogs (VAJDOVICH *et al.* 1997; SWANSON *et al.* 2004). According to these authors this can be explained by the shorter life span of erythrocytes and a lower haemoglobin concentration in the red blood cells of dogs before reaching sexual maturity.

In carnivorous animals the number of leukocytes in the blood is very variable and the changes concern both the total number of white blood cells and the relative percentage of their particular kinds (STANKIEWICZ 1973; BERESTOV & BRANDT 1989; MORITZ *et al*. 2004; WINNICKA 2004). The number of leukocytes in the blood of one-year-old females was 13.09 $G \cdot l^{-1}$ and was significantly ($P<0.05$) higher compared to the blood of females from the remaining age groups (8.23-9.11 $G \cdot l^{-1}$). Also, older females showed a significant ($P<0.05$) decrease in the percentage of lymphocytes and an increase in the relative content of granulocytes and monocytes in the blood, which may suggest a decrease in humoral immune response and may indicate the development of cell-mediated immunity with age. The conducted study did not show any significant age-dependent differences in the number of thrombocytes in the blood of experimental animals. The data agree with results of studies conducted on dogs and cats confirming a decrease in immunity with age (HEATON *et al.* 2002a, b; BRIEND-MARCHAL *et al.* 2003; BLOUNT *et al.*2005). The experiment of BLOUNT *et al.* (2005) on dogs aged from 2 to 10 years also showed a decrease in animal immunity with age,

Table 2

Mineral content and enzyme activities in the blood serum of polar fox females ($\bar{x} \pm$ SD); a, b, c – means in the rows with different letters differ significantly ($P<0.05$)

Parameters	Group				
	I	II	III	IV	V
Calcium, $mmol \cdot l^{-1}$	2.79 ± 0.05	2.76 ± 0.09	2.79 ± 0.09	2.77 ± 0.16	2.77 ± 0.10
Phosphorus, $mmol \cdot l^{-1}$	3.38^{a} ± 0.35	2.93^{b} ± 0.29	2.82^{b} ± 0.26	3.33^{a} ± 0.49	$3.17^{a,b}$ ± 0.43
Sodium, $mmol \cdot l^{-1}$	158.00 ± 1.63	158.10 ± 1.66	157.60 ± 1.90	159.00 ± 2.40	158.60 ± 2.41
Potassium, $mmol \cdot l^{-1}$	6.72^{a} ± 0.57	5.98^{b} ± 0.36	5.95^{b} ± 0.45	6.46^{ac} ± 0.56	6.06^{bc} ± 0.33
Chloride, $mmol \cdot l^{-1}$	114.00 ± 1.56	114.40 ± 1.26	112.80 ± 1.87	112.70 ± 1.95	113.50 ± 1.84
Magnesium, $mmol \cdot l^{-1}$	1.24 ± 0.10	1.17 ± 0.07	1.20 ± 0.09	1.23 ± 0.07	1.17 ± 0.07
Iron, $\mu mol \cdot l^{-1}$	39.49^{a} ± 6.73	38.10^{a} ± 9.59	$35.62^{a,b}$ ± 12.05	$31.85^{a,b}$ ± 4.12	28.87^{b} ± 6.63
Copper, $\mu mol \cdot l^{-1}$	12.48 ± 5.30	9.47 ± 0.89	10.45 ± 2.96	9.82 ± 0.64	9.90 ± 0.94
Zinc, $\mu mol \cdot l^{-1}$	20.58 ± 2.71	23.71 ± 8.55	26.14 ± 14.34	34.63 ± 11.74	20.98 ± 7.99
Alkaline phosphatase, $IU \cdot l^{-1}$	19.10 ± 4.86	25.00 ± 11.37	22.20±5.63	22.60 ± 5.44	22.10 ± 7.61
Acidic phosphatase, $IU \cdot l^{-1}$	14.90 ± 2.47	14.70 ± 2.67	12.90±1.97	12.40 ± 2.59	12.40 ± 2.80

which was manifested by a decrease in the absolute number of leukocytes (including lymphocytes, granulocytes and monocytes) in the peripheral blood. It was also observed that along with age there was an increase in the percentage of granulocytes and a decrease in percentage of lymphocytes in the total WBC content (CROOKS *et al.* 2000; HEATON *et al.* 2002a, b; BLOUNT *et al.* 2005). Despite a decrease in the absolute number of lymphocytes in the peripheral blood of dogs and cats, there was an increase in the percentage of T lymphocytes and a decrease in the percentage of B lymphocytes, which indicates the development of cell-mediated immunity with age (HEATON *et al.* 2002a, b; BLOUNT *et al.* 2005).

Table 2 shows the content of macro- and microelements and the activity of alcalic and acidic phosphatases in serum of polar fox females in relation to age. The concentration of Ca, Na, Cl and Mg in the blood was comparable in all animal groups and was within the physiological standards set for carnivorous animals (BENN *et al.* 1986; WINNICKA 2004). The highest content of Pi and K (3.38 and 6.72 $mmol \cdot l^{-1}$ respectively) was observed in the blood of one-year-old females. Two and three-year-old animals showed a significant ($P<0.05$) decrease in the level of these minerals (P: 2.82-2.93 $mmol \cdot l^{-1}$, K: 5.95-5.98 $mmol \cdot l^{-1}$), and then the concentration of Pi and K increased again in the blood of four and five-year-old females. In the present study, no significant age-dependent differences in activity of alcalic and acidic phosphatases were observed. Experiments conducted on various species of carnivorous and laboratory animals show a decrease in the content of Ca, Pi, K, Mg and ALP activity with age (CROOKS *et al.* 2000; TRYLAND *et al.* 2002; KLEY *et al.* 2003; SWANSON *et al.* 2004; COUDRAY *et al*. 2005). It is common knowledge that an increased level of alkaline phosphatase, calcium, and phosphorus in dog and fox puppies is related to the process of skeleton formation and osteoblast differentiation in growing animals (KASPAR & NORRIS 1977; CROOKS *et al.* 2000; KLEY *et al.* 2003). Polar foxes reach somatic maturity before 12 months of age, so in the experimental females the process of skeleton formation was complete. The iron content in the blood of experimental animals decreased with age and amounted to 39.49 and 28.87 $\mu mol \cdot l^{-1}$ ($P<0.05$) in one and five-year-old females, respectively. A decrease in content of mineral components in the blood of animals with age may be caused by various factors, including a decrease in absorption of nutrients and an increase in renal excretion (LEDGER *et al.* 1995; VAJDOVICH *et al.* 1997; HARPER 1998; COUDRAY *et al.* 2005). A decrease in iron content with age in females of the Beagle breed of dog was also noted by KASPAR & NORRIS (1977). This may be caused by a decrease in absorption of this nutrient from the alimentary tract and depends to a large extent on the iron status in the organism (AJIOKA *et al.* 2002). The available literature does not contain any data concerning the content of copper and zinc in the blood of polar foxes. In the present study, no significant differences in the level of these microelements were found. The Cu content oscillated between 9.47 and 12.48 $\mu mol \cdot l^{-1}$, and the Zn concentration between 20.58 and 34.63 $\mu mol \cdot l^{-1}$. A similar level of these microelements in peripheral blood of adult dogs of various populations of the Anatolian Shepherd breed was shown by ALTUNOK *et al.* (2001).

In conclusion, the present study shows that in the blood of adult female polar foxes in the nonmating period the content of red blood cells, haemoglobin and haematocrit index increases with age. In older animals the number of leukocytes and the percentage of lymphocytes decrease while the percentage of granulocytes and monocytes in the total number of white blood cells increases. Also the content of phosphorus, potassium and iron in the blood serum of females decreases with age. The available literature offers no reference values of haematological and biochemical blood parameters in the blood of polar foxes in relation to age and the subsequent reproductive season. The present study broadens the knowledge on blood variables from clinically healthy foxes for use by veterinarian and scientific investigators interpreting data determined by similar laboratory methods.

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